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Enantioselective Total Syntheses of Belactosin A, Belactosin C, and Its Homoanalogue†

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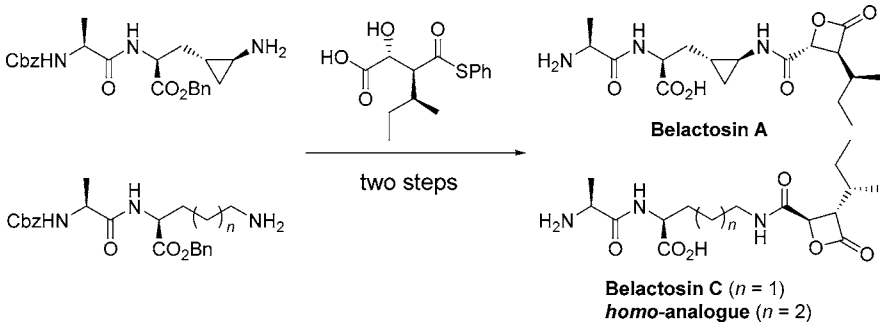
ABSTRACT



Enantioselective total syntheses of belactosin A, belactosin C, and its homoanalogue have been accomplished in high overall yields (32% for belactosin A from the amino acid 10, and 35 and 36% for belactosin C and its homoanalogue, respectively). This concise approach comprises a novel sequential acylation/*β*-lactonization reaction and allows a facile alteration of the substituents, thus providing a flexible route to a new family of highly active belactosin-based proteasome inhibitors.

Belactosins A (**1**) and C (**2a**) (Figure 1) were isolated from a fermentation broth of *Streptomyces* sp. UCK14 and exhibited antitumor activity,[1] which was shown to be increased significantly upon acetylation of the free amino group and esterification or amidation of the carboxyl group, as well as displacement of the ornithine moiety in **2a** with lysine to give **2b**, thus providing IC_{50} against human pancreoma and colon cancer on as low as the nanomolar level.[2] More intriguingly, the high antitumor activities of these derivatives appeared to be attributed to the proteasome inhibition.[3] This powerful mechanism of cell growth and

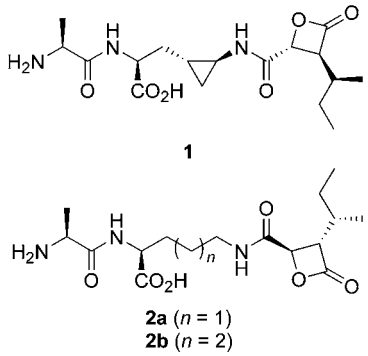


Figure 1. Belactosin A (**1**), belactosin C (**2a**), and its homoanalogue **2b**.

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† Part 99 in the series Cyclopropyl Building Blocks for Organic Synthesis. For Part 98, see: de Meijere, A.; Bagutski, V.; Zeuner, F.; Fischer, U. K.; Rheinberger, V.; Moszner, N. *Eur. J. Org. Chem.* **2004**, in press. For Part 97, see: Wiedemann, S.; Rauch, K.; Savchenko, A.; Marek, I.; de Meijere, A. *Eur. J. Org. Chem.* **2004**, 631-635.

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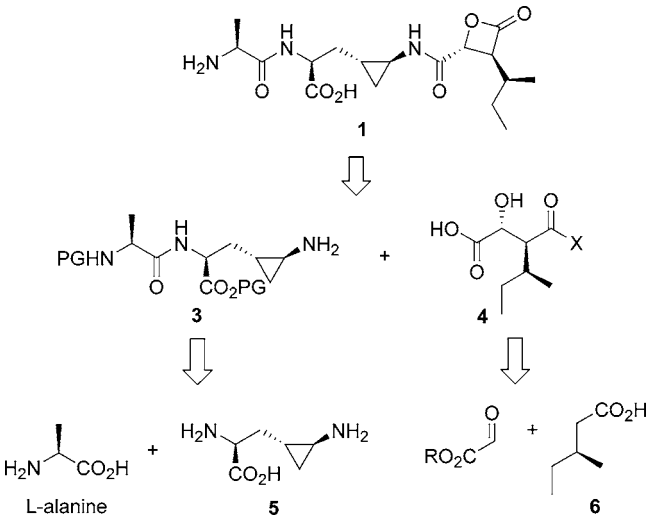
death control relies on the basic role of the proteasome in most cellular processes that are mediated by small peptide molecules produced by the proteasome.[3] Therefore, further studies of these substances exhibiting such remarkable biological activities[4] can ultimately lead to the development of new treatment options against cancer and inflammatory diseases.

In an effort to establish a flexible access to the belactosins and their derivatives, we embarked on a modular approach, starting from four distinct building blocks (Scheme 1). Their

Scheme 1. Retrosynthetic Analysis of Belactosin A

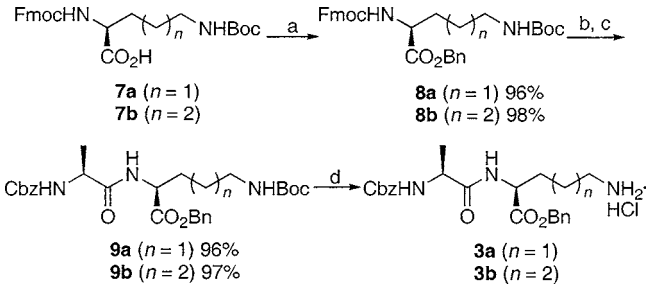


variation can give rise to a large set of analogues, and their efficient synthesis can provide a solid basis for further studies of their biological activities.

The belactosin compounds comprise an interesting trans-disubstituted β-lactone[5] with an adjacent stereogenic center in the side chain, thus calling for a special *anti*-selective aldol-addition strategy. Additionally, belactosin A contains the new cyclopropane amino acid *trans*-(2-aminocyclopropyl)alanine **5**, which has recently attracted considerable attention.[6] An additional challenge was to develop an approach that will tolerate a variety of substituents in the amino- and carboxyl-terminal amino acid moieties of the dipeptide **3**, thus implying that the final acetylation of the side chain amino group of **3** and subsequent β-lactone ring construction should be performed under very mild conditions.

First, the dipeptide components **3a** and **3b** were synthesized in four steps (Scheme 2). Benzylation of the commercial diprotected ornithine and lysine derivatives **7** with Cbz-Cl/DIEA/DMAP furnished the esters **8**, which after removal of the Fmoc group, were coupled with Cbz-Ala-OH. The Boc groups were cleaved off the resulting fully protected dipeptides **9** by treatment with 3 M hydrochloric acid to afford the hydrochlorides **3a** and **3b** in yields of 91 and 94%, respectively, over four steps.

Scheme 2. Synthesis of the Dipeptides **3a** and **3b**[a]



[a] Conditions: (a) CbzCl, DMAP, DIEA, CH_2Cl_2, 0 °C, 6 h; (b) Et_2NH, THF, rt, 3 h; (c) Cbz-Ala-OH, EDC, HOAt, TMP, CH_2Cl_2, 0 °C to rt, 16 h; (d) 3 M HCl in EtOAc, iPr$_3$SiH, rt, 17 h.

The attempted preparation of the diprotected *trans*-(2-aminocyclopropyl)alanine derivative **7c** as a prerequisite for the synthesis of belactosin A (**1**) by the recently published two-step general procedure,[7] which proved to be feasible for large-scale preparations of the analogous lysine and ornithine derivatives **7a** and **7b**, gave only a very low yield (27%) of **7c** (Scheme 2). This must be attributed to the experimental difficulties inevitably encountered on applying that procedure to subgram quantities of the enantiomerically pure *trans*-(2-aminocyclopropyl)alanine (**5**). To get around these difficulties, advantage was taken of the intrinsic differentiation of the two nitrogen atoms in *trans*-(2-nitrocyclopropyl)alanine (**10**), an established precursor to **5**.[6b] Therefore, compound **10** was converted into the respective Boc derivative **11** (84% yield). The nitro group in **11** was then reduced to an amino group. At first, this also presented a problem, since hydrogenation of **11** over Pd/C in methanol was shown to give rise to an extensive reductive cyclopropane ring cleavage.[8]

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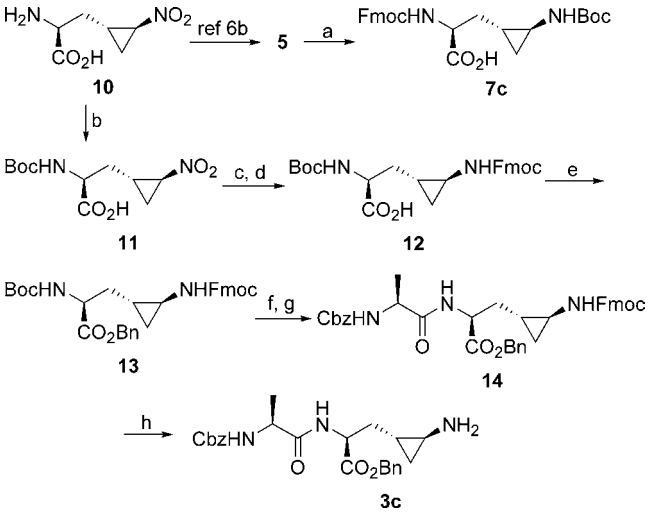
Scheme 3. Synthesis of the Dipeptide **3c**[a]

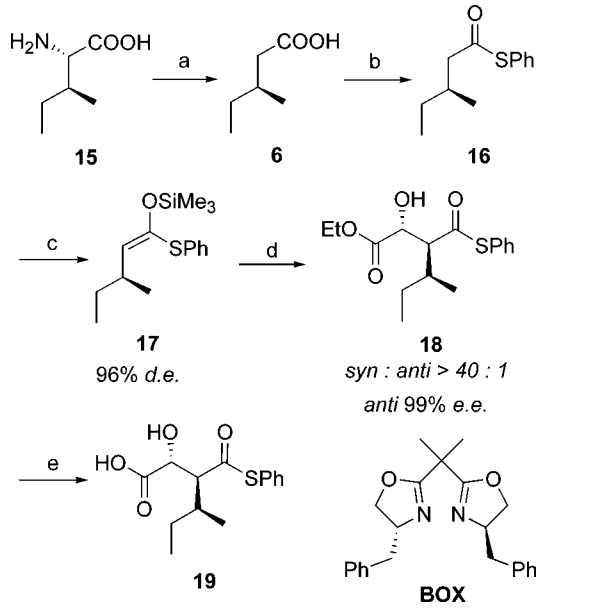


[a] Conditions: (a) (i) $CuSO_4$, $NaHCO_3$, Boc_2O, H_2O, Me_2CO, rt, 48 h; (ii) Fmoc-OSu, 8-quinolinol, Na_2CO_3, rt, 3 h, 27% (over two steps). (b) Boc_2O, Na_2CO_3, 6 N aq KOH, aq dioxane, rt then 35 °C, 30 h, 98%. (c) Zn, AcOH, rt, 3 h. (d) Fmoc-OSu, Na_2CO_3, H_2O, DMF, rt, 3 h, 75% (over two steps). (e) Cbz-Cl, DMAP, DIEA, CH_2Cl_2, 0 °C, 6 h, 81%. (f) TFA, *i*Pr_3SiH, CH_2Cl_2, rt, 4 h. (g) Cbz-Ala-OH, EDC, HOAt, TMP, CH_2Cl_2, 0 °C to rt, 16 h, 94% (over two steps). (h) Et_2NH, THF, rt, 3 h.

This overhydrogenation proved to be difficult to circumvent even by employing more basic (e.g., DMF) or less polar (e.g., EtOAc) solvents. On the other hand, the reduction of an oxime containing a cyclopropane ring, to an amine, could be efficiently accomplished by Zn dust in AcOH, even when the hydrogenation led to reductive ring destruction.[9] Gratifyingly, the reduction of the nitro group in **11** with Zn in AcOH proceeded cleanly, to give the aminocyclopropyl intermediate,[10] which was immediately transformed into the Fmoc-protected derivative **12** (75% over two steps). This underwent smooth benzylation at the carboxyl group to give the ester **13**, which, after acid-catalyzed removal of the Boc group, was in turn coupled with Cbz-Ala-OH (94% over two steps). The Fmoc group in the dipeptide **14** was then cleaved off by treatment with diethylamine to give the desired amino dipeptide **3c** with a terminal amino group.

Considering different approaches to the β-lactone component **4**, the necessity to establish the *anti* configuration along with that of the adjacent side chain stereogenic center, bearing substituents of a very similar size (i.e., methyl and ethyl), had to be kept in mind. This makes the construction of the remote stereogenic center via common synthetic protocols unfavorable. Employing L-isoleucine (**15**) allows all the merits of a chiral pool compound as a starting material to be enjoyed. L-Isoleucine (**15**) was hydrodeaminated[11] with hydroxylamine-*O*-sulfonic acid in 84% yield (Scheme 4).

Scheme 4. Synthesis of the β-Lactone Building Block[a]



[a] Conditions: (a) H_2NOSO_3H, NaOH, H_2O, 0 °C, 6 h, reflux, 3 h, 84%; (b) DCC, PhSH, DMAP, CH_2Cl_2, 0 °C to rt, 7 h, 97%; (c) LiTMP, Me_3SiCl, THF, −78 °C, 16 h, 92%; (d) EtO_2CCHO, $Sn(OTf)_2$ (10 mol %)/**BOX** (11 mol %), CH_2Cl_2, −78 °C, 20 h, then 2 N HCl, THF, rt, 2 h, 99%; (e) 10% aq HCl, dioxane (1:6), 60 °C, 51 h, 74%.

With the acid **6** in hand, the construction of **4** was eventually accomplished. A number of noncatalytic anti-selective aldol reaction protocols were tested, but they provided neither satisfying yields nor diastereo- and enantioselectivities. Attention was therefore turned to the Mukaiyama-type protocol for an efficient catalytic asymmetric aldol reaction as developed by Evans et al.[12] The acid **6** was converted into the phenyl thioester **16**, which was subsequently transformed into the (*Z*)-silylketene acetal **17**. This was then employed in the Mukaiyama-type aldol reaction with ethyl glyoxalate, using $Sn(OTf)_2$/**BOX** as the catalytic system. This procedure afforded the substituted malic acid derivative **18** in an excellent yield (99%) and with a high degree of enantio- (99% ee) and diastereoselectivity (*syn*:*anti* > 40:1)[13] even on a multigram scale.

To be able to proceed with the assembly of the belactosin backbone, the ethyl ester group in **18** had to be cleaved without affecting the phenyl thioester moiety. This did not

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(10) Another successful example of selective reduction of nitro- to aminocyclopropanes by Zn in HCl has recently been described: Wurz, R. P.; Charette, A. B. *J. Org. Chem.* **2004**, *69*, 1262−1269.

(11) Doldouras, G. A.; Kollonitsch, J. *J. Am. Chem. Soc.* **1978**, *4*, 341−342.

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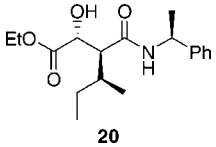
(13) Absolute configuration of **18** was unambiguously established by an X-ray crystallographic analysis of the amide **20**, which was prepared from **18** and L-phenylethylamine.



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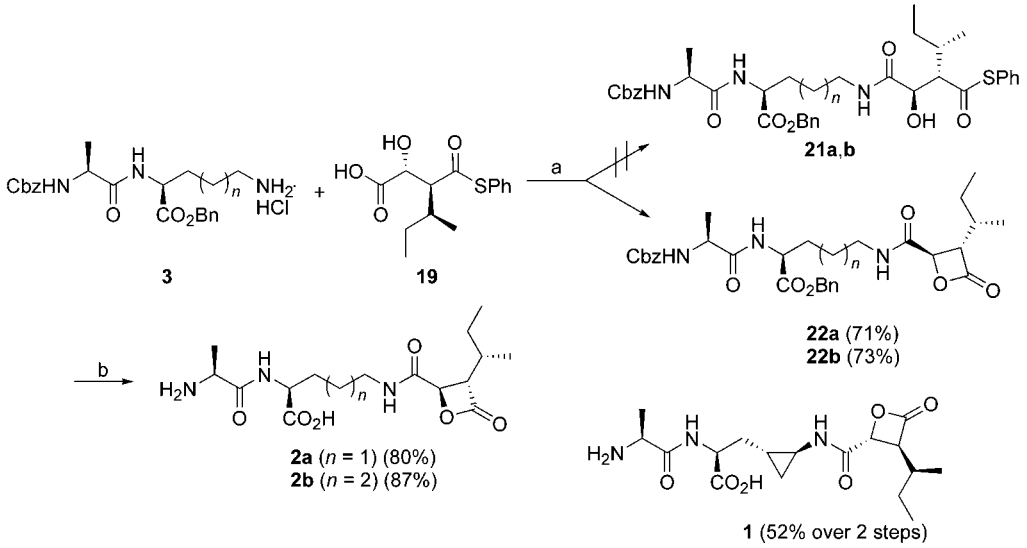
For details, see: Larionov, O. V.; de Meijere, A.; Yufit, D. S.; Howard, J. A. K. *Acta Crystallogr. E* **2004**, *60*, o681-o683.

Scheme 5. Final Assembly of the Belactosin Backbone[a]



[a] Conditions: (a) EDC, HOAt, TMP, CH_2Cl_2, −30 °C, 6 h, rt, 30 h, 74%; (b) H_2 (1 atm), Pd/C, AcOH, rt, 15 h.

appear to be trivial, since in the majority of described cases, thioester groups usually are highly selectively hydrolyzed in the presence of *O*-ester groups. An attempted enzymatic hydrolysis of the ethyl ester group of **18** under neutral conditions completely failed, probably due to the high hydrophobicity of the molecule. Fortunately, the desired selective ethyl ester cleavage succeeded under acidic conditions (10% aq HCl in dioxane at 60 °C), furnishing the acid **19** in 74% yield. It is noteworthy that strict temperature control (i.e., ±3 °C) is required to achieve a high yield, since at lower temperatures the hydrolysis is too slow, whereas at higher temperatures retro-aldol decomposition occurs.

Subsequent condensations of the acid **19** with the dipeptides **3a**,**b** were envisaged to yield the thioesters **21** (Scheme 5), which were then supposed to give the β-lactone products **22** upon activation with thiophilic metal salts (Cu^I, Ag^I, Hg^{II}),[14] or, if this would fail, hydrolytic removal of the phenylthio group, followed by β-lactonization, to furnish the desired diprotected belactosin derivatives **22a**,**b**. Quite unexpectedly, the reaction of **19** with **3a** under the peptide coupling conditions (EDC/TMP/HOAt) directly led to the β-lactone products **22** in 62−73% yields. This appears to be the first example of such a domino-type acylation/β-lactonization sequence. However, this reaction may actually follow a more complicated pathway than just acylation with subsequent β-lactone ring closure, since an attempt to effect the β-lactonization of **18** under the same conditions failed. A more detailed study of this transformation is currently underway. The benzyl and Cbz groups of the β-lactone compounds **22** were then cleaved off by hydrogenolysis in AcOH, thus affording the three members **1** and **2a**,**b** of the belactosin family.

In conclusion, we have accomplished the first enantioselective total syntheses of belactosin C (**2a**) and its homoanalogue (**2b**), along with a new total synthesis of belactosin A (**1**). Our convergent approach comprises only 11 steps (overall yields of 35 and 36%, respectively) in the cases of **2a** and **2b** and 14 steps (overall yield of 32% from **10**) for **1** and involves a new domino-type acylation/β-lactonization sequence, which may be employed in syntheses of further β-lactone carboxamides, derived from carboxyl-substituted β-hydroxy acids.

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Supporting Information Available: Experimental procedures and full characterization for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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